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LABORATORY INVESTIGATION

PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys

CHARLES E. ALPERS, RONALD A. SEIFERT, KELLY L. HUDKINS, RICHARD J. JOHNSON, and DANIEL F. BOWEN-POPE

Departments of Pathology and Medicine, University of Washington School of Medicine, Seattle, Washington, USA

PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys. There is evidence that platelet derived growth factor (PDGF) is a mediator of proliferative changes in renal arteries and mesangium in human disease, in the mesangium in experimental mesangial proliferative glomerulonephritis, and in the interstitium in a rodent model of angiotensin II mediated hypertension. We utilized a monoclonal antibody to the β-subunit of the PDGF-receptor to localize constitutive expression of this receptor in human and nonhuman primate tissues. Tissues were fixed in cold 2 or 4% paraformaldehyde, and immunohistochemical techniques both at the light microscopic level and immunoelectron microscopy were employed. In the glomerulus, there is widespread expression of this molecule by mesangial cells, and there is frequent expression on the apical and lateral surface of parietal epithelial cells. There is also widespread expression of this molecule by cortical and medullary peritubular interstitial cells, but not by glomerular or peritubular capillary endothelium or other renal parenchymal structures. The identification of receptors capable of binding PDGF B-chain at each of these sites: (1) provides a basis for PDGF mediated mesangial proliferation in human disease; (2) provides a basis for PDGF mediated interstitial cell migration and/or proliferation and/or activation at sites of tubulointerstitial injury; and (3) suggests that glomerular parietal epithelial cells may be responsive to stimulation by PDGF.

Platelet-derived growth factor (PDGF) mediates multiple cellular activities in a variety of cell types via specific receptors. These activities include stimulation of cell proliferation [1, 2], stimulation of cell migration, particularly of mesenchymal cells such as smooth muscle cells and fibroblasts [3–7], and induction of connective tissue matrix synthesis and deposition both *in vitro* and in the course of wound healing [8–11]. Platelet-derived growth factor is a covalent dimer of two subunit chains designated A-chain and B-chain, which exists in three naturally occurring dimeric isoforms (PDGF-AA, PDGF-AB, and PDGF-BB). PDGF binds to cells by cell surface receptors which function as noncovalent dimers of two subunits, designated as PDGF receptor α-subunit (PDGFRα) and the PDGF receptor β-subunit (PDGFRβ), in such a way that cells expressing only PDGFRβ are able to bind only PDGF-BB and cells expressing PDGFRα are able to bind all forms of PDGF [12].

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Recent studies have provided evidence that PDGF B-chain has an important role both in experimental and human nephritis. In the rat model of mesangial proliferative glomerulonephritis induced with anti-Thy1 antibody, up-regulated synthesis of PDGF B-chain and the PDGF-β receptor by mesangial cells occurs at times of peak cell proliferation [13, 14] and the cell proliferation occurring at day four of this model can be significantly reduced by administration of a neutralizing anti-PDGF B-chain antiserum [15]. Up-regulation of PDGF A- and B-chain mRNA has also been demonstrated in a murine model of dextran induced IgA nephropathy [16]. Immunohistochemical evidence for participation of PDGF in human disease has been provided by studies which have localized PDGF to mesangial areas in IgA nephropathy and other mesangial proliferative glomerulonephridities [16, 17], and by studies of expression of PDGF-receptor in which the receptor expression appears to have been upregulated in arterial vessels involved in transplant rejection as well as in glomeruli in cases of mesangial proliferative glomerulonephritis [18].

Although PDGFR expression in normal human kidneys has been reported, the exact cellular localization of these PDGFRs in normal human kidney has not been well defined. Fellström detected weak mesangial expression of PDGFRβ in 3/6 normal human kidneys by immunohistologic techniques on frozen tissue sections, and apparently found no normal staining within the cortical interstitium or other glomerular structures [18]. In a survey study of frozen human tissues, Franklin et al found mesangial localization of PDGFRβ in eight kidneys [19], but the immunocytochemical approaches reported to date do not allow more precise assignment of expression of this protein to specific cell types, particularly within the interstitium. In the present study, we have utilized a well characterized monoclonal antibody to PDGFRβ to localize its expression in normal human and primate kidneys. We demonstrate by both immunohistologic and, for the first time, immunoelectron microscopic techniques that PDGFRβ is constitutively expressed by mesangial cells and parietal epithelial cells in the glomeruli of humans, macaques, and baboons. We also show widespread constitutive expression of PDGFRβ by cortical and medullary interstitial cells. These latter findings provide evidence that these interstitial cells are likely to be responsive to PDGF, which may thus mediate at least in part the migration and activation of these cells at sites of interstitial injury and fibrosis.

Methods

Source of tissue

Normal human kidney tissue ($N = 25$) was obtained fresh from uninvolved portions of kidneys surgically resected for localized renal cell carcinoma, or from cadaver donor kidneys unable to be utilized for transplantation. Normal primate kidneys, baboon ($N = 4$) and *macaca nemestrina* ($N = 6$), were obtained fresh from the Regional Primate Research Center at the University of Washington. The tissue was fixed overnight in cold 2% or 4% paraformaldehyde in phosphate buffer, transferred to 30% sucrose in 0.1 M phosphate buffer, equilibrated overnight at 4°C, and snap frozen in OCT compound (Miles, Inc., Elkhart, Indiana, USA).

Immunohistochemistry

Murine monoclonal antibody PR7212 has been previously characterized by Western blotting and competitive binding studies and shown to recognize the β subunit of the PDGF receptor [20]. The epitope recognized by this antibody is stable in paraformaldehyde fixed tissues [21].

Frozen sections of 2% or 4% paraformaldehyde fixed tissue were hydrated in PBS and then incubated overnight at 4°C with antibody 7212, washed, and processed using streptavidin-biotin immunoperoxidase method with biotinylated horse-anti-mouse IgG (Vector Labs, Burlingame, California, USA) as the secondary antibody, Vectastain Elite ABC (Vector Labs) as the detection system and 3,3′-diaminobenzidine (DAB) as the chromogen. Endogenous peroxidase was blocked by incubating the slides in 3% hydrogen peroxide following incubation with the biotinylated secondary antibody. The sections were counterstained with methyl green, dehydrated and coverslipped.

For all samples, a negative control consisted of substitution of the primary antibody with both irrelevant isotype-matched murine monoclonal antibodies and PBS.

Immunoelectronmicroscopy

Frozen, 4% paraformaldehyde fixed kidneys were sectioned at 6 μm and sections adhered to APTS coated slides and air dried for 30 minutes. The sections were then stained by hydrating in PBS for 15 minutes, incubated in 0.05% sodium borohydride in PBS at 4°C for 60 minutes to reduce free aldehyde groups, rinsed and then incubated with antibody PR7212 or control antibody at 4 μg/ml in PBS containing 2% BSA overnight at 4°C. The slides were then processed as above using the streptavidin-biotin immunoperoxidase method. After washing in distilled water, sections were reacted with 2% OsO_4 for one hour at room temperature, rinsed and then dehydrated through graded ethanols and into propylene oxide. Sections were then infiltrated with a 50/50 mixture of PolyBed (Poly-Sciences, Inc, Warrington, Pennsylvania, USA) and propylene oxide for one hour. Beem capsules were filled with PolyBed, inverted over the sections, infiltrated overnight and then polymerized at 55°C for 48 hours. The blocks were removed by heating the slide briefly and quickly snapping off the capsule. Thin, 0.1 micron sections were cut and mounted on grids and examined in a Philips 410 electron microscope.

Results

Immunohistochemistry

Results of the immunohistochemical studies in baboons, macaques, and humans are identical and will be described together. In all cases, there is diffuse expression of PDGFRβ within mesangial areas (Fig. 1A, B). Expression of PDGFRβ by parietal epithelial cells was also frequently detected (Fig. 1A). There was no evidence of PDGFRβ expression by visceral epithelial cells, cells of the juxtaglomerular apparatus, or hilar vasculature.

There was also widespread expression of PDGFRβ by cells within the cortical and medullary interstitium (Fig. 1B–D). Arterial vessels showed no detectable expression of PDGFRβ by either endothelial cells or smooth muscle cells (Fig. 1C). Prominent staining of arterial adventitial connective tissue cells was observed. Tubular cells were uniformly negative.

At this level of fixation and resolution, required for preservation of the epitope recognized by the 7212 antibody, it is not possible to clearly differentiate expression by endothelial cells lining the peritubular capillaries from expression by interstitial fibroblasts.

Immunoelectron microscopy

Glomeruli. Immunoelectron microscopy confirmed immunohistochemical studies demonstrating that PDGFRβ expression is confined to the mesangium and parietal epithelial cells (Figs. 2, 3). There is no extension of reactivity to the monoclonal antibody to basement membranes of the peripheral capillaries walls, or to visceral epithelial cells. There is peroxidase product indicative of PDGFRβ expression at spaces where mesangial cells about mesangial channels and there is accordingly peroxidase product in those areas where mesangial cells are exposed to the capillary lumen. In those sites, one cannot absolutely exclude regional expression of PDGFRβ by endothelial cells. However, in each case where the cell borders of endothelial cells overlying these regions can be distinguished from underlying mesangial cells, the expression of PDGFRβ can be clearly seen to be confined to the mesangial cells (Fig. 2). In no case was PDGFRβ expression identified in the fenestrated portions of endothelial cells lining the peripheral capillary loops.

Immunolocalization of PDGFRβ expression at the ultrastructural level also revealed uniform, distinct staining of the apical, and at times lateral, cell surface of parietal epithelial cells (Fig. 3). No evidence of expression at the basal cell surface was identified.

Interstitium. Human tissue fixed in glutaraldehyde and prepared for transmission electron microscopy by conventional techniques is used to illustrate the typical appearance of cortical interstitial cells, as seen in Figure 4. Human cortical interstitial cells, like those described in rodents [22, 23], are characterized by their long, thin cell processes, focally prominent rough endoplasmic reticulum, the absence of other distinctive cell organelles, and the absence of cell junctions. They are without obvious attachment to tubular basement membranes, tubular epithelial cells, or the peritubular capillaries, although the processes at times appear to extend in close proximity to the tubular basement membranes.

Immunoelectron microscopy shows that it is this interstitial cell type and not peritubular capillary endothelium, tubular

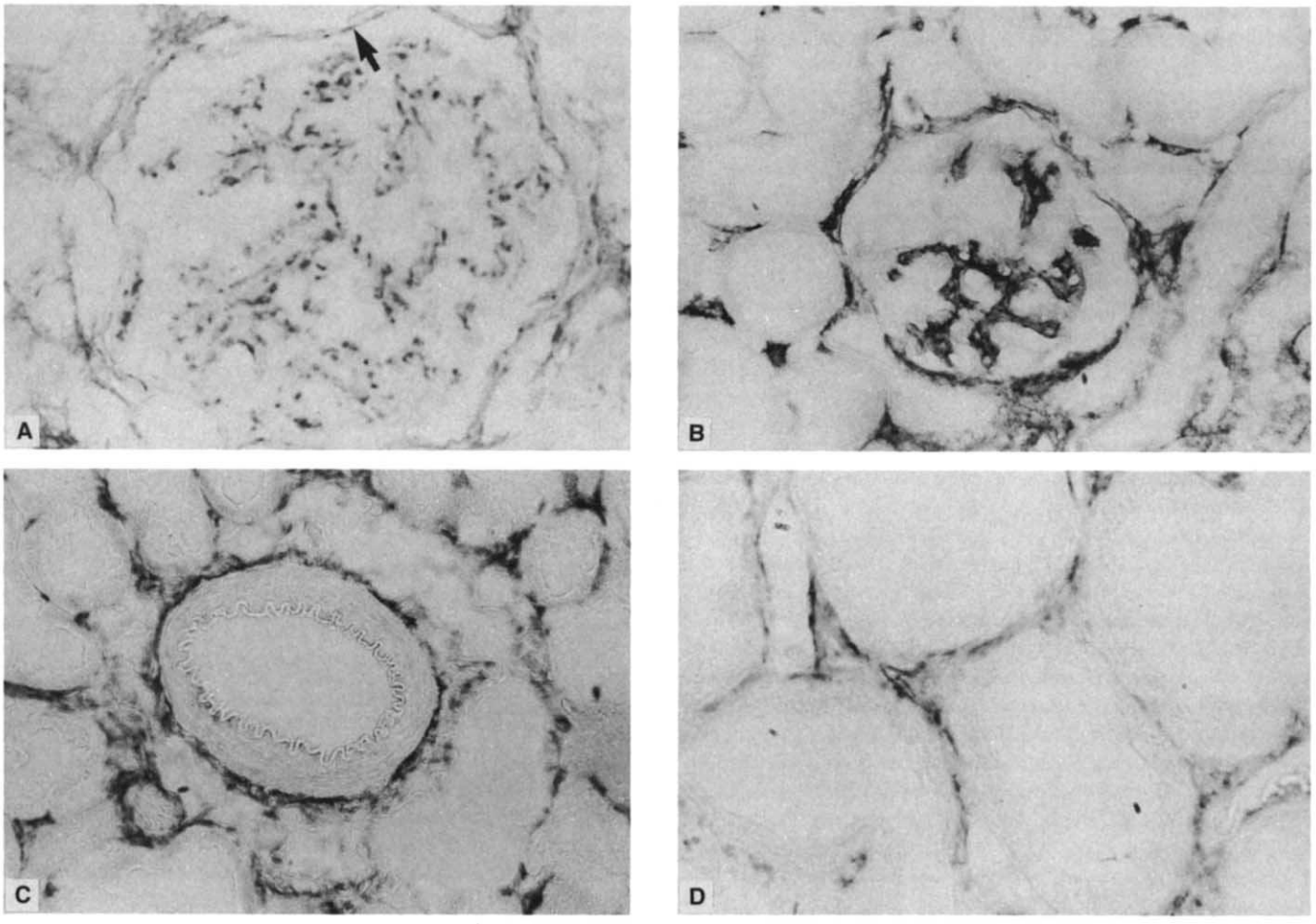

Fig. 1. A. *Human kidney.* PDGFRβ expression in the glomerulus is typically present diffusely within the mesangium, and can also be seen focally on the apical surface of parietal epithelial cells (arrow). Surrounding interstitium contains a population of PDGFRβ expressing cells. 480×. **B.** Baboon kidney. There is striking localization of PDGFRβ to mesangial areas. Glomeruli in subhuman primates normally demonstrate the wider mesangial regions seen here, as compared with human kidneys. Widespread expression of PDGFRβ by cortical interstitial cells is also apparent. 240×. **C.** Baboon kidney. Arcuate artery shows no detectable expression of PDGFRβ by endothelial cells or smooth muscle cells comprising the vessel intima and media. A population of adventitial connective tissue cells is strongly positive, as are the adjacent interstitial cells. 240×. **D.** Human medulla. The interstitium shows diffuse expression of PDGFRβ by dark staining spindled cells throughout the intertubular space. Adjacent tubular epithelium is consistently negative. In some areas, PDGFRβ expressing cells can be localized to regions of the interstitium located between peritubular capillaries and tubular segments. 960×.

structures, or occasional interstitial leukocytes, which regularly express PDGFRβ (Fig. 5). Our studies indicate the presence PDGFRβ diffusely with no evidence of polar distribution of the receptor in these cells.

Controls. Substitution of an irrelevant isotype matched monoclonal antibody as well as PBS for primary antibody PR7212 abolished all specific staining (Fig. 6).

Discussion

It has been shown that mesangial cells *in vitro* are responsive to PDGF, which can induce mesangial cell proliferation, migration, synthesis of matrix components, and stimulate further production of this peptide by the mesangial cells themselves [2, 13–15, 24, 25]. Rat mesangial cells *in vivo* will also proliferate in response to exogenous infusion of PDGF (J. Floege, et al, unpublished observations). Human mesangial cells *in vitro* have been shown to express PDGFRβ, although some levels of PDGF α receptor can also be demonstrated [26]. In addition, PDGF and PDGFRβ are expressed in mesangial regions in both experimental and human mesangial proliferative glomerulonephritis [16–18], and inhibition of PDGF with anti-PDGF antibody in a rat model of acute, severe mesangiolytic injury reduced the subsequent cell proliferation [15]. This has suggested that human mesangial cells *in vivo* express physiologically significant levels of the PDGF receptor. This study demonstrates the unequivocal localization of PDGFRβ to normal human and nonhuman primate mesangial cells, and so provides further evidence in support of the premise that mesangial binding of PDGF is an important part of the response to glomerular injury in humans.

This precise localization is consistent with the apparent mesangial localization of PDGFRβ expression previously observed by light microscopy on frozen sections of human kidney [19]. However, because of the limited resolution afforded by studies of unfixed tissue, those studies could not clearly distinguish mesangial from endothelial expression in the glomerulus.

Fig. 2. *Immunoelectron microscopy of a human glomerulus.* PDGFRβ expression is confined to the mesangium, (M) while glomerular capillary endothelium (E) and visceral epithelial cells demonstrate no expression. 5150×.

This is an important issue because in some organs, such as the brain, it has been suggested that PDGFRβ may be expressed by some capillary endothelial cells [27, 28]. In this study, the use of immunoelectron microscopy establishes that it is the mesangial cell exclusively that constitutively expresses PDGFRβ. Our studies found no evidence of PDGFRβ expression by the endothelial cells of the glomerulus. This finding provides a logical extension of our studies of human fetal kidney, where we have shown that expression of PDGFRβ is uniformly present in the mesangium of differentiated glomeruli [21]. Unlike some other proteins apparently produced by mesangial cells in human kidneys *in utero*, such as α smooth muscle actin, PDGF B-chain, and possibly Factor VIII related antigen, which appear to undergo extensive downregulation prior to maturity [21], PDGFRβ expression remains readily detectable by immunohistochemical techniques. In contrast to the studies of Fellström et al [18], this study indicates that mature human mesangial cells express detectable levels of the PDGFRb in histologic sections prior to induction of any disease state; it would then appear that this basal expression of the PDGF receptor may be upregulated in disease settings. Based on our understanding of rodent models of glomerular immune and nonimmune glomerular injury [13, 25], and the suggestive findings of Fellström et al [18], it is likely that modulation of PDGFRβ expression is an important mediator of human glomerulopathy.

A second finding of interest is the demonstration of PDGFRβ expression at the surface of glomerular parietal epithelial cells. Little is known about the functional activities of these cells, and their interactions with other cells of the glomerulus is an area that is largely unexplored. Recently, our group has shown that visceral epithelial cells in the rat produce PDGF B-chain but not PDGFRβ as a response to cell injury induced by passive Heymann nephritis [29]. Some proliferation of parietal epithelial cells was noted in that study, although the parietal epithelial cell is not thought to be a direct target of immune injury in that model. Those findings, and the present demonstration of PDGFRβ on parietal epithelial cells, suggest a potential interaction between glomerular epithelial cells that is mediated by local, paracrine release of PDGF B-chain by visceral epithelial cells and binding of this peptide to its receptor on parietal epithelial cells.

The constitutive expression of PDGFRβ by normal human and primate renal interstitial cells is the third principal finding of this study. There are currently no known specific cellular markers of renal interstitial cells that distinguish such cells from other mesenchymal cells within the kidney parenchyma. This study suggests PDGFRβ may be such a marker *in vivo*. As in the mesangium, this observation is an important extension of our observations in human fetal kidneys, where it was shown that a diffuse population of mesenchymal interstitial cells express PDGFRβ [21]. In the mature kidney, PDGFRβ expression is widespread and generally uniform along the cell surface of those interstitial cells having features of fibroblasts, that is, with spindled cell shape, focally prominent rough endoplasmic reticulum and absent cell attachment structures. One result of this rather uniform staining of cell borders is that the immunohistochemical and immunoelectron microscopic studies illustrate to a degree greater than can be appreciated by unmodified transmission electron microscopy the extent to which the cell bodies of interstitial fibroblasts extend and course through the interstitial parenchyma, often in parallel with the peritubular capillaries. This immunoelectron microscopic localization of PDGFRβ to the interstitial cells rather than peritubular capillaries in this study might be considered a somewhat unexpected finding that contrasts with evidence from brain tissue which points to capillary endothelium as the site of PDGFRβ expression [27, 28].

What role PDGFRβ expression might be playing in tubulointerstitial biology or disease is at present highly speculative. Much less is known about the activities of growth factors within the interstitium as compared with the glomerulus, and there are currently no disease models of tubulo-interstitial injury in which a role for PDGFRβ has been tested and established. However, in angiotensin II mediated injury in rats, interstitial fibrosis, type IV collagen deposition and tubular cell proliferation has been associated with increased PDGF expression in interstitial areas [30]. In this model the associated increase in PDGF expression was also correlated with phenotypic changes of interstitial cells which demonstrated α smooth muscle actin expression and proliferation of a proportion of these actin-expressing cells [30].

Despite the current paucity of relevant disease models, but given what is known of PDGF biology, some investigators have already offered hypotheses that link PDGF to renal interstitial fibrogenesis [31–33]. Additional evidence to support a role for PDGF in this type of response to injury comes from studies of

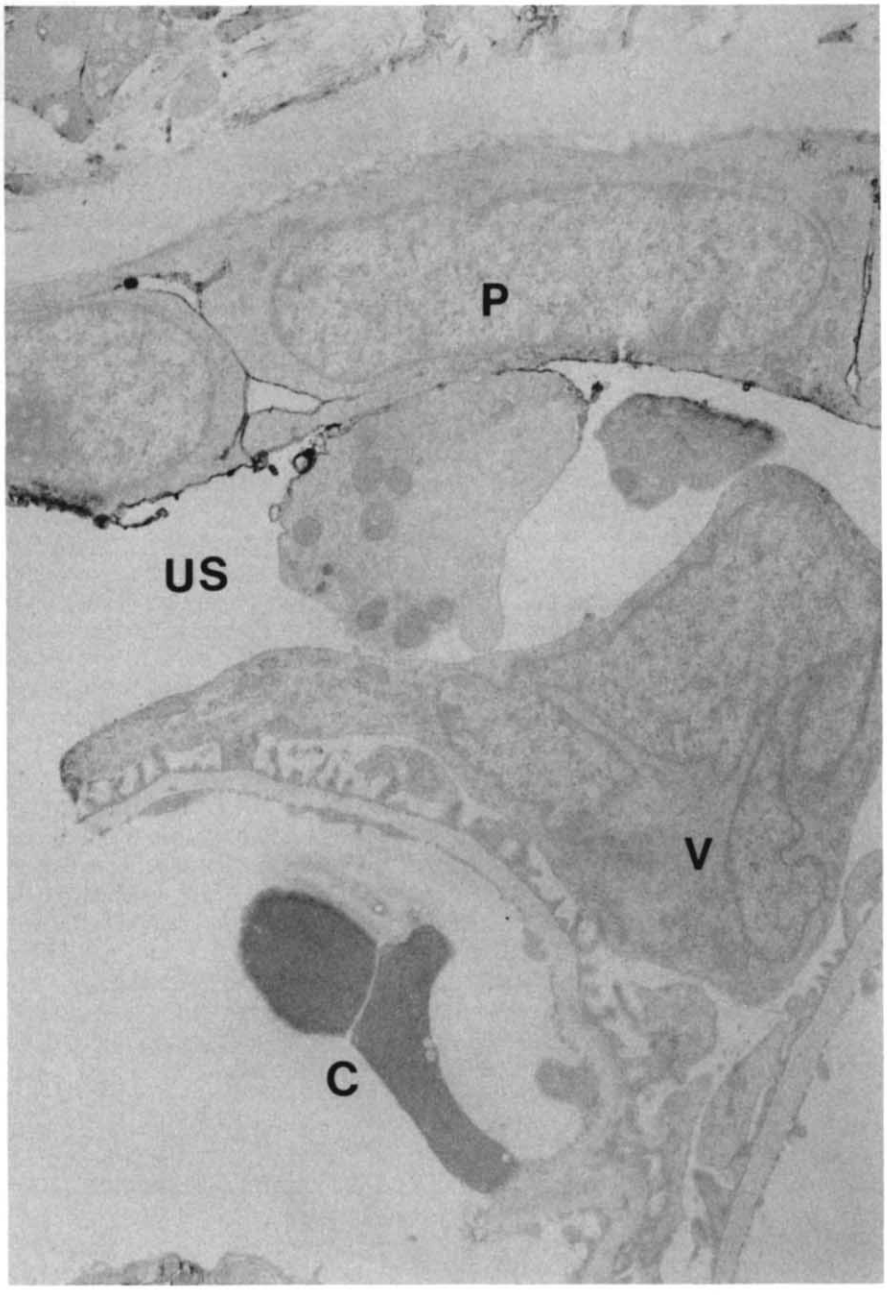

Fig. 3. *Macaque kidney.* PDGFRβ is expressed at apical and lateral cell surfaces of parietal epithelial cells (P). Visceral epithelial cells (V) and their foot processes, and capillary endothelium show no expression. Disruption of the capillary basement membrane is a sectioning artifact, where the capillary wall has left the plane of section. Portions of a dislodged parietal epithelial cell are present in the urinary space (US). Abbreviation: C, capillary lumen. 6050×.

wound healing in other sites such as the skin, where PDGF responsive cells, presumably expressing PDGFR, can accelerate the repair process [9–11]. Preliminary studies in our laboratory suggest that PDGFRβ expressing cells can accumulate in sites of tubulo-interstitial injury, but it has not yet been possible to ascertain whether these cells migrate from other portions of the renal interstitium, migrate from other organs via the circulation, or represent a locally derived, proliferating population of cells (C.E. Alpers, unpublished observations).

In this regard, it has been increasingly accepted that the extent of tubulo-interstitial scarring is the principle determinant of residual renal functional reserve in human parenchymal renal disease, regardless of whether the glomerular, vascular, or tubulo-interstitial compartments were the primary sites of injury [31–34]. Little is known about the specific mechanisms by which fibroblasts and other mesenchymal cells may migrate to or proliferate at sites of interstitial fibrosing injury and then participate in this process of matrix accumulation and scarring. Constitutive expression of PDGFRβ by interstitial cells, cells that have been identified as "cortical fibroblasts" or cortical "type one cells" by others [22, 23], suggests a potential mechanism by which these cells can be recruited to sites of injury. It is possible that release of PDGF, perhaps by infiltrating cells such as monocytes/macrophages and platelets which are known to localize in areas of tubulo-interstitial injury [30, 35–38], or alternately by adjacent tubular epithelial cells [39], serves as a stimulus to recruit and possibly activate interstitial fibroblasts at these areas of injury.

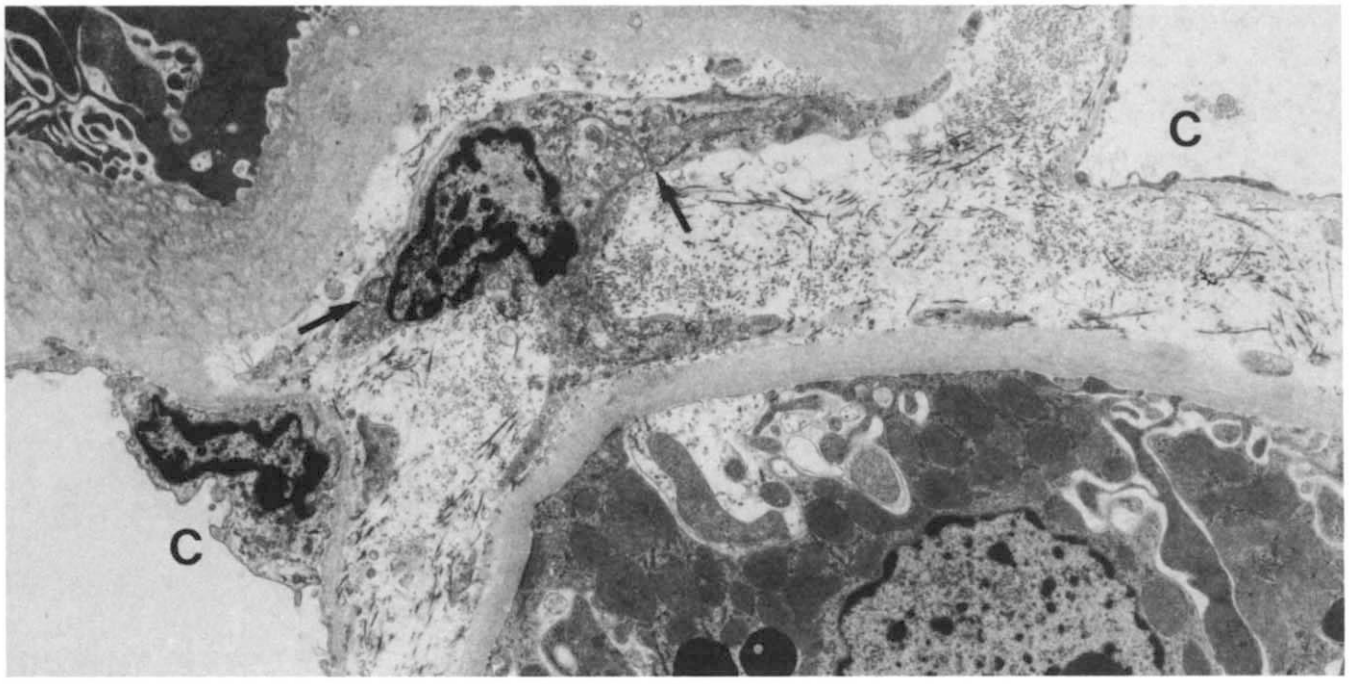

Fig. 4. *Human kidney.* Transmission electron micrograph of the tubulo-interstitium showing tubular epithelial cells with prominent mitochondria and interdigitating baso-lateral membranes, peritubular capillaries (C) lined by endothelium, and a central, spindled cortical interstitial cell with prominent rough endoplasmic reticulum (arrows) and absent cell junctions. 6150×.

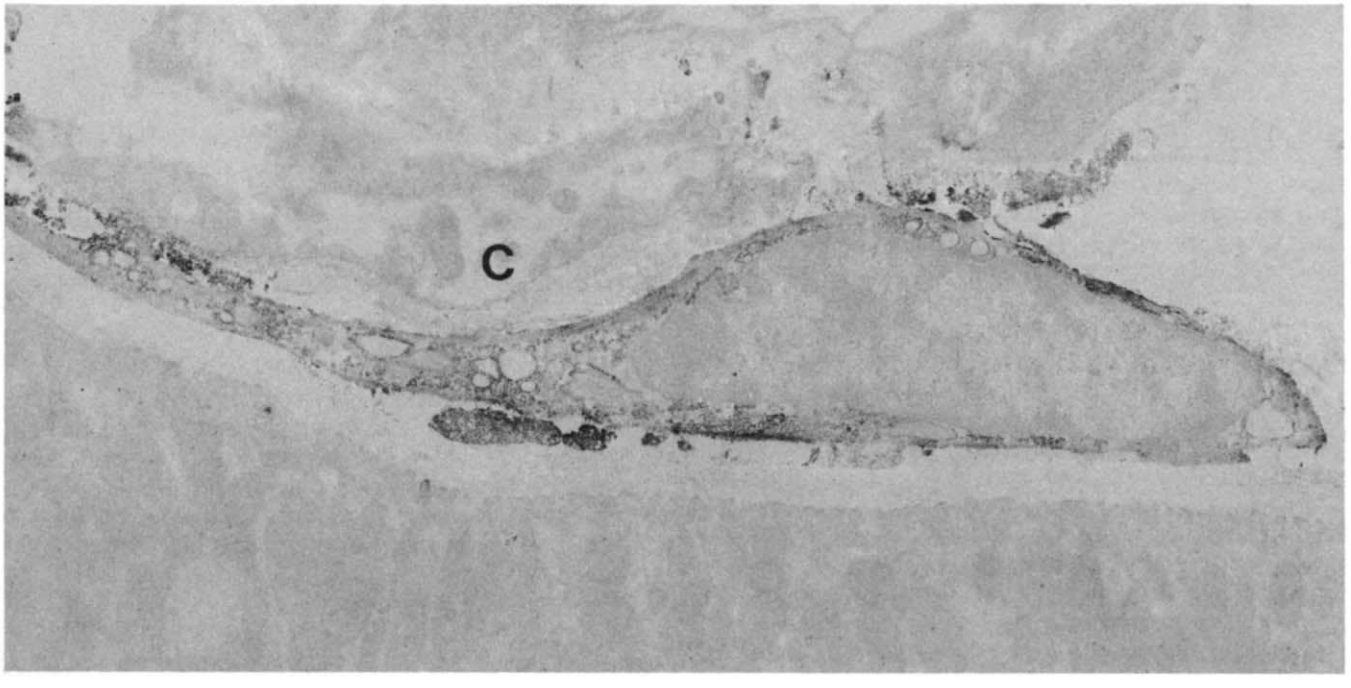

Fig. 5. *Macaque kidney.* PDGFRβ is expressed diffusely by a cortical interstitial cell like that illustrated in Figure 4, while adjacent collagen matrix and capillary endothelium show no expression of the receptor. Abbreviation: C, Capillary. 8600×.

One surprising and important observation in this study was the failure to demonstrate PDGFRβ expression by smooth muscle cells in normal renal arteries. Observations *in vitro* indicate that vascular smooth cells are responsive to PDGF B-chain, and therefore should express PDGFRβ [1]. The studies of Fellström et al, are of particular interest in that up-regulated expression of PDGFRβ in injured renal arteries was demonstrated [18]. Taken together, the evidence suggests

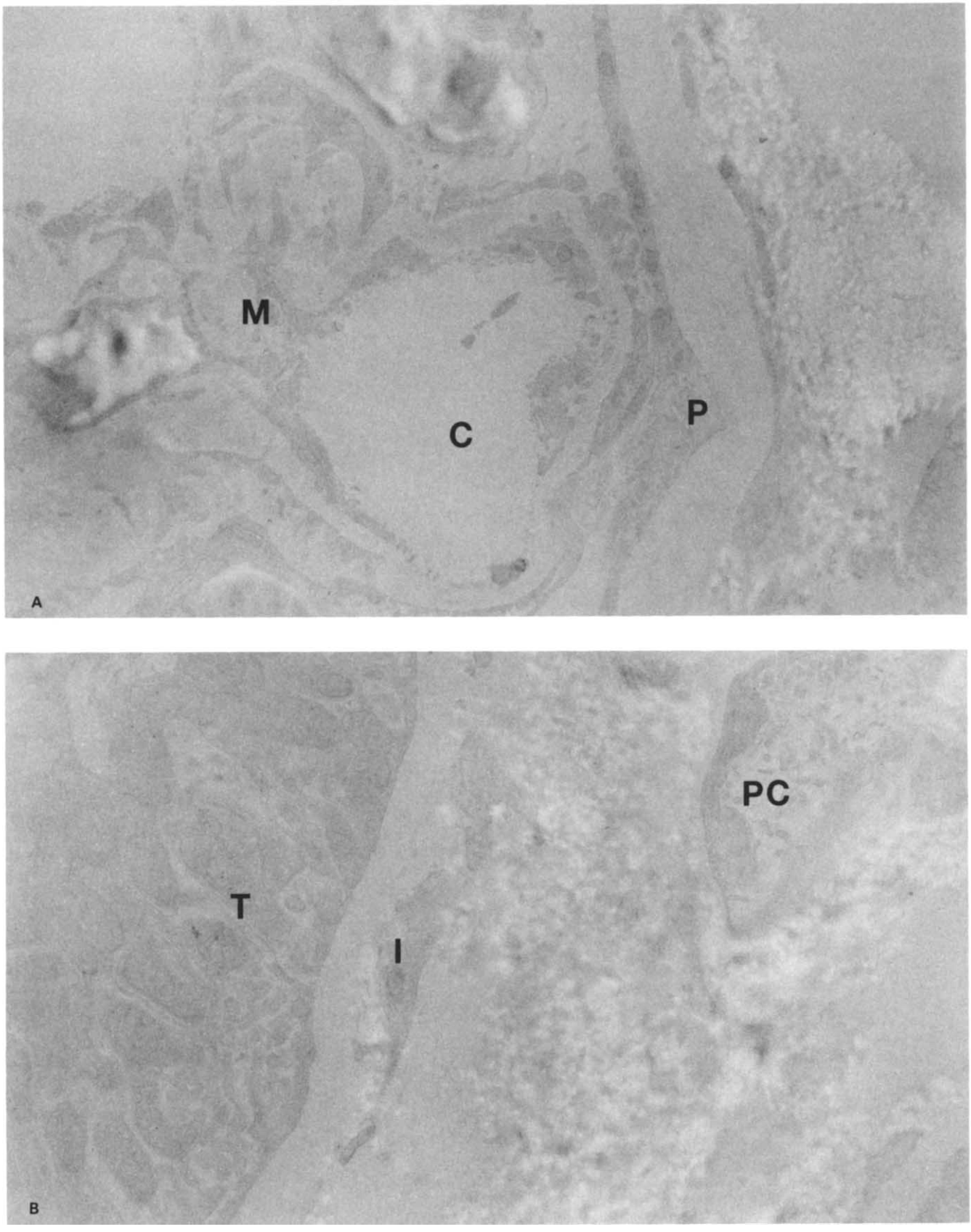

Fig. 6. *Human kidney.* Substitution of an irrelevant monoclonal antibody for the primary antibody used in Figures 1 through 5 shows absence of specific staining of mesangial cells and parietal epithelial cells **(A)** and interstitial cells **(B).** Abbreviations are: M, Mesangium; C, glomerular capillary lumen; P, parietal epithelial cell; T, tubule; I, interstitial cell; PC, peritubular capillary. **A:** 5800×. **B:** 9100×.

PDGF B-chain may mediate part of the arterial response to injury, while a trophic function for this growth factor in undamaged portions of the vasculature is unlikely. This study did not address renal expression of PDGF a receptor, which may be important in vascular smooth muscle cells, because reagents suitable to address this area in fixed tissue are not yet available.

Methods to obtain populations of renal interstitial cells from rabbits and humans and maintain them in culture have recently been reported [40, 41]. Some of these studies indicate rabbit medullary fibroblasts, but not cortical fibroblasts, may be responsive to PDGF [40]; this stands in contrast to this study which indicates human and primate cortical and medullary fibroblasts are likely to be responsive to PDGF. When cultured human renal interstitial cells become more widely characterized and available, it should be possible to investigate the responsiveness of these cells to PDGF as well as other migration and growth regulatory molecules and cytokines. Understanding these relationships may eventually allow development of therapeutic strategies that might interrupt processes of progressive interstitial injury, akin to recent successful approaches to ameliorate specific growth factor effects in experimental glomerulonephritis [15, 42].

Acknowledgments

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Reprint requests to Charles E. Alpers, M.D., Department of Pathology, RC-72, University of Washington Medical Center, Seattle, Washington 98195, USA.

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